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EARLY CRETACEOUS PHLEBOTOMINE SAND FLY LARVAE (DIPTERA: PSYCHODIDAE)

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Abstract.—Two sand fly larvae (Diptera: Psychodidae) are characterized from Early Cretaceous Burmese amber. Both larvae, which are considered to be post-first instars, possess only a single pair of caudal setae on the terminal (9th) abdominal segment. All known extant post-first instar sand fly larvae possess two pairs of caudal setae except for the Old World *Phlebotomus (Larroussius) tobbi* Adler and Theodor and New World *Brumptomyia* França and Parrot. The fossil larvae could represent an ancestral line continued today by *P. tobbi* or a completely separate lineage, and they show that a single pair of caudal setae was an ancient characteristic. A close association of the fossil larvae with the fruiting bodies of a non-gilled coral fungus, *Palaeoclavaria burmitis* Poinar and Brown (Hymenomycetes: Palaeoclavariaceae), suggests a source of nourishment for Early Cretaceous sand flies.

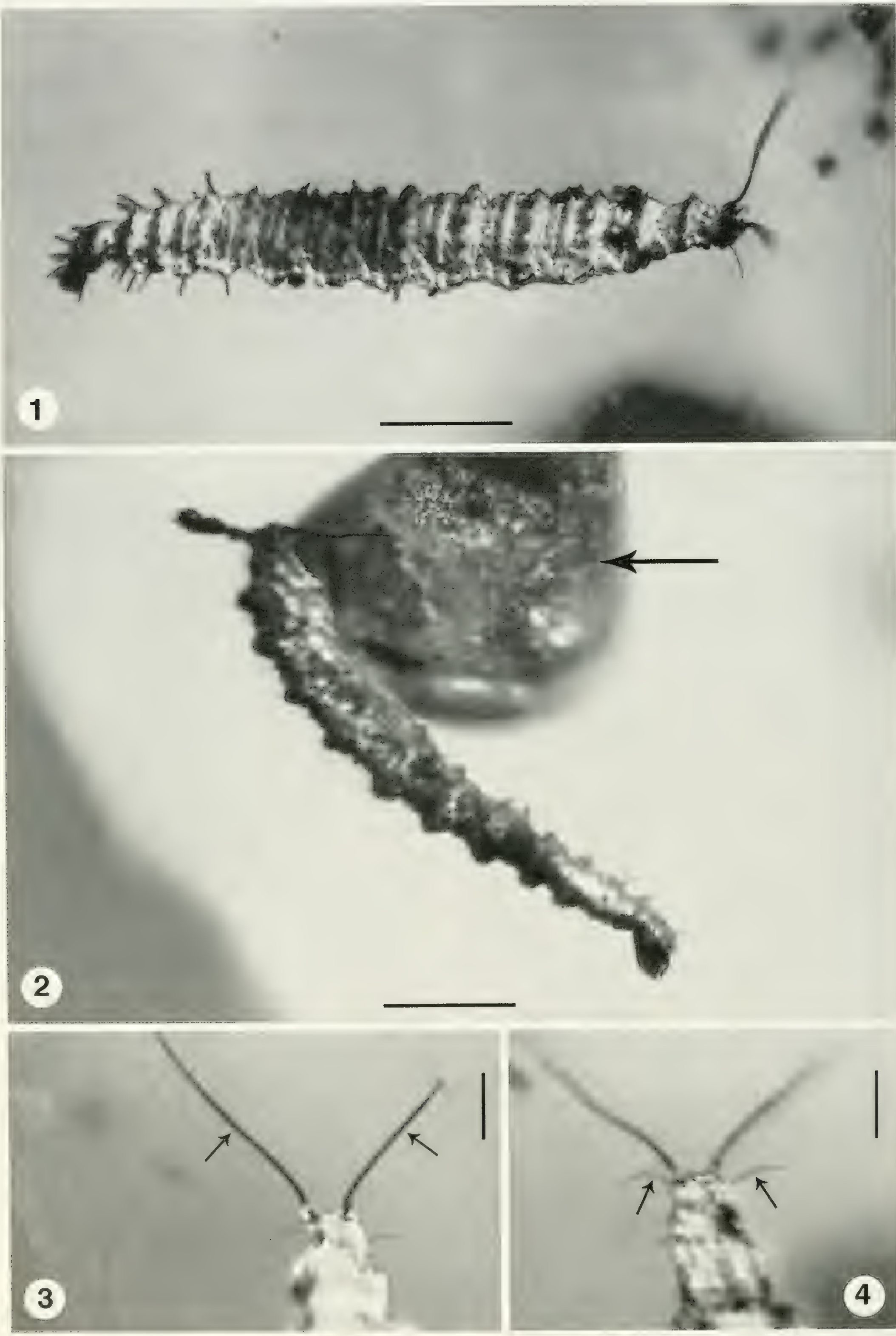
Key Words: sand fly larvae, Early Cretaceous, caudal setae, food source, fossil fungus, Burmese amber

During a survey of hematophagous insects in amber, a piece of Early Cretaceous amber from Myanmar (Burma) was discovered to contain a pair of sand fly larvae together with their possible fungal food source (Figs. 1, 2). These larvae are characterized, and their morphological features compared with those of extant sand fly larvae. This is the first fossil record of sand fly larvae.

Materials and Methods

The amber piece containing the fossil sand fly larvae was received in the summer of 2004 from the Leeward Capital Corp., a Calgary-based company working with a Burmese mining company in Noije Bum. The amber with the fossils is semicircular in outline, measuring 13 mm along the longest side, 9 mm wide, 4 mm deep and weighs 0.4 gm. Observations, drawings and photographs were made with a Nikon SMZ-10 R stereoscopic microscope and Nikon Optiphot compound microscope with magnifications up to 600×. The amber piece containing the fossil flies is deposited in the Poinar amber collection (accession #B-D-24) maintained at Oregon State University.

Amber from Burma occurs in lignitic seams in sandstone-limestone deposits in the Hukawng Valley, southwest of Maingkhwan in the state of Kachin (26°20′N, 96°36′E). Palynomorphs obtained from the amber beds have been assigned to the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Upper Albian (~ 100–105 mya) of the Early Cretaceous (Cruickshank and Ko 2003), however since the amber is secondarily deposited, the age could be older. Nuclear magnetic resonance (NMR) spectra of amber samples taken from the same locality as the fossils indicated an araucarian (Araucariaceae) (possibly *Agathis*) as a possible source of the amber (J. B. Lambert and Y. Wu, unpublished data, 2002).

Results

Both sand fly larvae are similar in size and have the basic characters typical of phlebotomine larvae (Quate and Vockeroth 1981, Perfil'ev 1966), such as a hypognathous head, 3 thoracic and 9 abdominal segments, oval third antennal segments and long, paired caudal setae on the last abdominal segment (Figs. 1–6). The first two thoracic segments bear small pseudopods (tubercles) on their ventral surfaces, the last thoracic and first 8 abdominal segments bear prominent pseudopods and the last abdominal segment has a small pseudopod. The larva nearest the edge of the amber (No. 1) (Fig. 1) is 2.77 mm in length and has an approximate head capsule length of 214 μm. The larva adjacent to a fungal fruiting body (No. 2) (Fig. 2) is 2.83 mm in length and has an approximate head capsule length of 220 μm. Only approximate head capsule lengths could be obtained since both larvae have their heads slightly turned making definite measurements impossible. There is no evidence that the larvae are shrunken or distorted.

Both larvae are uniformly cream colored and have light head capsules. However, the ventral pseudopods on the thoracic and abdominal segments appear as darker areas. The antennae are located near the front border of the head (Fig. 5). Only the oval-oblong third segment of the antenna is visible, and from this segment projects a minute, blunt- tipped apical spine. Depression lines extend through these terminal segments. The whorled spinules on the setae are quite reduced and many appear to be absent with only the ringlike bases remaining. The mouthparts are obscured.

Both larvae possess only one pair of caudal setae arising from the dorsum of the terminal (9^{th}) abdominal segment (Figs. 1–6). These setae vary from 0.725 to 0.910 mm in length (the tip of the left caudal seta on larva 1 was polished away). A pair of shorter spinulose setae (0.16 to 0.21 mm in length) is located on the last abdominal segment. This pair extends outward and slightly downward from a subdorsal position beneath and outside the caudal setae. There are no other noticeable setae on the last abdominal segment.

The amber piece also contains several fruiting bodies of the coral mushroom, *Palaeoclavaria burmitis* Poinar and Brown, a member of the non-gilled Aphyllophorales (Hymenomycetes). The posterior portion of larva No. 2 is attached to a fungal fruiting body by a mucilaginous-appearing substance. Another fruiting body has what appears to be an isolated piece of fungal tissue adjacent to a hole in its side. Both larvae

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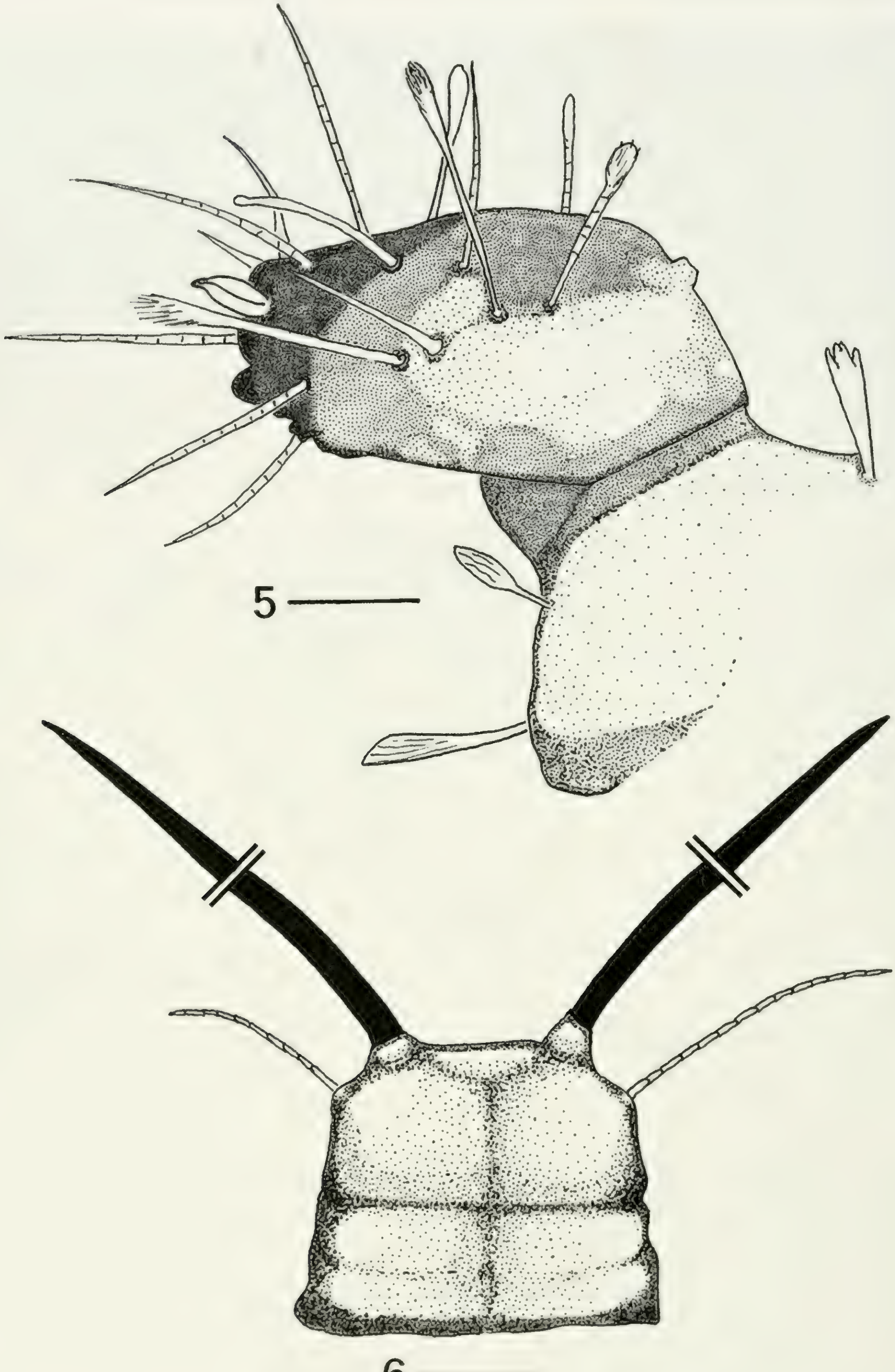
Figs. 1–4. Early Cretaceous sand fly larvae. 1, Dorsal-lateral view of larva No. 1. Bar = 503 μm. 2, Lateral view of larva No. 2. Adjacent to the larva is its likely food source, a fruiting body of the coral fungus, *Palaeoclavaria burmitis* (arrow). Bar = 608 μm. 3, Dorsal view of last (9^{th}) abdominal segment showing paired caudal setae (arrows). Bar = 200 μm. 4, Dorsal view of last (9^{th}) abdominal segment showing paired spinulose setae (arrows). Bar = 200 μm.



Figs. 5–6. Early Cretaceous sand fly larva (camera-lucida line drawings). 5, Lateral posterior view of head of larva No. 1 showing antenna and arrangement and types of cephalic setae. Bar = 50 μm. 6, Dorsal view of last (9th) abdominal segment showing paired inner caudal setae and paired outer spinulose setae. Bar = 8 μm.

have small particles of what could be fungal debris attached to their bodies. Voided fecal pellets occur at the terminus of both larvae. A mite (Acari) is the only other arthropod inclusion in the amber. Mites would be expected in such a habitat and some are ectoparasites of sand flies (Warburg et al. 1991).

Discussion

The generic identity of the fossil sand fly larvae is unknown, but on the basis of size and morphology, both probably belong to the same stage and same species. Thus far, *Palaeomyia burmitis* Poinar (2004) is the only sand fly described from Burmese amber, and while the larvae could belong to this species, they could also be from undescribed species from that amber source. It is difficult to determine the instar of the fossil larvae since Early Cretaceous sand flies are smaller than extant Old World species (the Burmese amber mines are located on the Burma Plate (Mitchell 1993, Cruickshank and Ko 2003) which in the Early Cretaceous, was part of Laurasia, thus the fossil larvae belong to Old World clades).

Adult lengths of some Early Cretaceous sand flies and closely related psychodid flies are 0.887 mm and 1.04 mm for the Burmese amber *Palaeomyia burmitis* Poinar (2004) and *Eophlebotomus connectens* (Cockerell) (Duckhouse 2000), respectively, and 1.06 mm, 1.07 mm, 0.93 mm, and 1.05 mm for the Lebanese amber *Mesophlebotomites hennigi* Azar et al. (1999), *Libanophlebotomus lutfallahi* Azar et al. (1999), *Phlebotomites brevifilis* Hennig (1972), and *Eophlebotomus gezei* Azar et al. (2003), respectively. The two specimens of *Eophlebotomus carentonensis* Azar et al. from Early Cretaceous French amber have lengths of 0.93 and 0.97 mm (Azar et al. 2003).

The Cretaceous adult lengths (0.89–1.07 mm) are smaller than those of extant Old World adults, which range from 1.20 mm to 3.70 mm (Perfil'ev 1966) (Table 1). The Cretaceous fossils are also smaller than the New World Dominican amber phlebotomine fossils *Trichopygomyia killickorum* Filho et al. (2004) (adult length = 1.58 mm) and *Pintoyia falcaorum* Brazil and Filho (2002) (adult length = 1.46 mm). Assuming that the size of the adults is correlated with those of the larvae, then the lengths of the larval stages of the fossil species likely would be smaller than the corresponding sizes of extant larvae. A comparison of the lengths of the fossil larvae with those of extant larvae of *Phlebotomus (Phlebotomus) papatasi* Scopoli, *P. (Adlerius) arabicus* Theodor, *Sergentomyia (Sergentomyia) bedfordi* Newstead, *S. (Sergentomyia) hamoni* Abonnenc, *S. (Rondaniomyia) ingrami* Newstead, and *S. (Rondaniomyia) dureni* Parrot (Table 1) shows that they are definitely post-first instars and possibly even fourth instars since their lengths overlap with those of extant fourth stage larvae. Extant first stage sand fly larvae have rounded heads with an almost spherical third antennal segment (Perfil'ev 1966). The fossil larvae have elongated heads and the third antennal segment is oblong-oval (Fig. 5), which is typical of 2nd to 4th larval instars. Both the body and head of the fossil larvae are light colored, which is similar to the yellow color of some fourth stage *Lutzomyia* França larvae (Ward 1976). The third instars of *Lutzomyia (Psychodopygus) panamensis* (Shannon) also have light-colored head capsules similar to those of the fossil larvae (Lawyer and Perkins 2000). Voiding the gut contents just before pupation also gives the body a whitish appearance (Lawyer and Perkins 2000).

An interesting feature of the fossil larvae is the presence of only one pair of caudal setae on the terminal abdominal segment. The only known extant sand fly

Table 1. Lengths (mm) of larval instars and adults of some extant *Phlebotomus* and *Sergentomyia* sand flies.

Species	1st Stage	2nd Stage	3rd Stage	4th Stage	Adult	Reference
P. arabicus	0.6–1.2	1.4–2.5	2.3–3.4	3.3–4.6	2.5–3.0	Volf[1,2]
P. papatasi	0.5–1.0	1.3–1.4	—	—	2.5–3.2	Perfil'ev, 1966
P. papatasi	0.4–0.8	2.3–2.8	3.2–3.5	3.6–4.2	2.4–3.3	R.L. Jacobson[2]
S. bedfordi	0.3–0.7	0.8–1.0	—	2.1–2.8	1.8–2.4	Trouillet, 1977[1]
S. ingrami	0.9–1.0	—	—	2.2–2.6	1.6–2.4	Trouillet, 1979[1]
S. dureni	0.6–1.0	—	—	2.2–2.6	1.6–2.4	Trouillet, 1979[1]
S. hamoni	0.5–0.6	—	—	2.3–2.6	2.3–2.6	Trouillet, 1979[1]

[1] Adult measurements taken from Abonnenc (1972).
[2] Unpublished observations.

larvae which possess a single pair of caudal setae in the post-first instars is *Phlebotomus tobbi* from the Ionian Greek islands and Syria (Killick-Kendrick et al. 1989, 1992). However, *P. tobbi* has four downwardly directed spinulose setae on the ventral surface of the ninth segment, which differs from the single pair of subdorsal spinulose setae in the fossil larvae. Mature larvae of New World *Brumptomyia* França and Parrot species have two caudal setae (Theodor 1965, Young and Duncan 1994) while *Lutzomyia* larvae have two pairs of caudal setae on post-first instars (Ward 1976, Quate and Vockeroth 1981, Lawyer and Perkins 2000). Some mormiine moth fly larvae (Psychodinae) possess a pair of caudal filaments but they are shorter and differ from sand fly setae (Jezek 1989).

Reduction is generally the rule in the evolutionary process. Therefore, Early Cretaceous Old World post-first instar sand fly larvae would be expected to possess at least two, if not more pairs, of caudal setae. The fossils could represent an ancient Old World lineage continued today by *P. tobii* (Killick-Kendrick et al. 1989, 1992), and though this species is absent from Myanmar today, the subgenus, *Larroussius* occurs in the region (Lewis 1978). However, the fossil could represent a completely separate now extinct lineage.

The fruiting bodies of the coral fungi associated with the fossil larvae have been described as *Palaeoclavaria burmitis* (Hymenomycetes: Palaeoclavariaceae) (Poinar and Brown 2003). A perforation in one of the fruiting bodies adjacent to some extruded fungal debris and the close association of larva No. 2 to a second fruiting body suggests an intimate relationship between the larvae and the fungus. It is possible that the sand fly eggs were deposited on or close to the fruiting body, which then served as both a source of humidity and food for the eclosed larvae. This fungus probably developed around wounds or in rotting portions of the trunk or branches of the resin-producing tree, which is one of the developmental habitats of extant sand flies (Hanson 1961, Rutledge and Mosses 1972). It is possible that these, as well as the closed sporocarps of other fungi, were a food source for Early Cretaceous sand flies. Today some fungi, especially those with closed fruiting bodies, are important developmental habitats for primitive flies such as fungus gnats (Mycetophilidae and Sciaridae), gall midges (Cecidomyiidae), crane flies (Tipulidae), scavenger flies (Scatopsidae), and wood gnats (Anisopodidae) (Bruns 1984). Fungi as food for extant sand flies is mentioned by Perfil'ev (1966) and Wermelinger and Zanuncio (2001). In a study of the larval feeding habitats of Panamanian sand flies, the preferred sites were sheltered areas between tree buttresses, which were also the resting habitat for adult sand flies (Hanson 1961). The substrate in these areas was composed of organic matter from plant and animal remains. It is possible that the sand fly larvae feed on fungi developing in and on this organic matter.

The small size of the Early Cretaceous sand flies may indicate an early period in the establishment of adult and larval feeding niches. If fungal fruiting bodies were an important food source for Early Cretaceous sand flies, then their occurrence would have been seasonally variable and unpredictable, which would favor an increased feeding rate and reduced developmental times. The caudal setae may have been partially exposed on the outside of the fruiting body, possibly serving as detectors of approaching danger since recent studies suggest that they have a sensory function (Pessoa et al. 2001).

Just when phlebotomine sand flies evolved is still unresolved. The oldest known fossils occur in 120 million year old Lebanese amber (Azar et al. 1999); however, it is not known whether these were biting forms since descriptions are based only on males. The oldest evidence of a haematophagous habit in sand flies is in the Burmese amber *Palaeomyia burmitis* that contains within its body not only leishmanial parasites but also infected vertebrate blood cells (Poinar and Poinar 2004a, b).

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